Diffusion-limited Reduction of Chromium in Soil Aggregates

Tetsu K.Tokunaga*, Jiamin Wan*, Terry C. Hazen*, Egbert Schwartz†, Mary K. Firestone†, Stephen R. Sutton‡, Matthew Newville‡, Keith R. Olson*, Antonio Lanzirotti‡, William Rao§

*Lawrence Berkeley National Laboratory, Berkeley, California, 94720 USA
†University of California, Berkeley, California 94720, USA
‡University of Chicago, Chicago, IL 60637, USA
§Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA

Industrial development has resulted in a long history of environmental contamination[1,2]. The fate of metal contaminants in soils and sediments is controlled by interdependent influences of transport and biogeochemical reactions. The gap between understanding of simple batch systems and observations on complex natural subsurface systems motivates examining biogeochemical dynamics in systems of intermediate complexity, individual soil aggregates. Soil aggregates (cohesive units comprised of individual mineral particles) can contain wide variations in chemical[3,4] and microbiological[5,6] composition, especially when intra-aggregate gradients in redox potentials exist (Fig. 1). We examined influences of aggregate microenvironments and microbial communities that control the fate of Cr(VI) contamination. Reduction of Cr(VI) to Cr(III) occurred only within the surface layer of aggregates with higher available organic carbon and higher microbial respiration. Sharply terminated Cr diffusion fronts develop when the reduction rate increases rapidly with depth. The final state of such aggregates consists of a Cr-contaminated exterior, and an uncontaminated core, each having different microbial community compositions and activity. Analogous behavior may be expected for other redox-sensitive transformations in soils and sediments.

Chromium is used in a variety of industrial processes, and its hexavalent species have become a serious problem as a soil contaminant at hundreds of hazardous waste sites because it is a respiratory carcinogen, and acutely toxic in high doses[7]. In uncontaminated soils, Cr is found as Cr(III) because of the high pe range associated with Cr(VI)-Cr(III) transformations, and high proportions of Cr(VI) are largely restricted to contaminated sites[8,9]. Cr(III) occurs in soil primarily as stable solids and strongly adsorbed species, while most Cr(VI) species are soluble and mobile. The soil microbial community can promote Cr(VI) reduction to Cr(III) directly through enzymatic pathways[10,11], and indirectly through depleting oxygen and releasing reductants such as ferrous iron, sulfide, and organic compounds[12,13]. Recent comparisons of abiotic versus enzymatic Cr(VI) reduction kinetics indicate that aqueous Fe(II) is expected to be dominant in neutral to alkaline anaerobic soils[14,15]. Active microbial communities nevertheless exert dominant influences since they mediate the depletion of oxygen and the availability of reactive reductants (Fe(II) and S(-II)). Soil microbial communities are also influenced by exposure to high concentrations of Cr(VI), with responses including death, resistance development, and enzymatic reduction[10,16]. Thus, interactions between soil microbial communities and invading Cr(VI) solutions are complex but central to the fate of the contaminant.

We present two types of experiments on Cr redox zonation. Experiments were conducted on small soil columns designed to represent transects into synthetic aggregates[17], and on actual soil aggregates. Under more reducing conditions in the small column experiments (redox potentials < -100 mV), Cr(VI) diffusion and Cr(III) precipitation was limited to distances of a few mm, with abruptly terminated transport fronts (Fig. 2A). Thus, sharp boundaries between contaminated and uncontaminated regions can develop. Principal component analysis of terminal restriction fragment length polymorphism (TRFLP) patterns[18] distinguished the region exposed to Cr (VI) from unexposed regions (Figure 2B). Ribosomal Intergenic Spacer Analysis of extracted bacterial DNA also showed that unique intergenic sequences were present in the soil sample exposed to Cr(VI). Control tests on Cr(VI) in sterile soils with carbon added showed negligible Cr reduction rates.

With higher microbial activity, anoxic conditions are established at shallower depths. Such conditions facilitate Cr(VI) reduction within short distances below oxic surfaces, thereby sustaining large Cr(VI) concentration gradients at the surface, and larger diffusive influxes. The sharply terminated Cr contaminant fronts indicate that Cr(VI) reduction rates increase rapidly with depth into the aggregates. The measured average total Cr depth profile (local depth averaged Cr concentrations from Fig. 2A map) is compared with finite-difference calculations of Cr diffusion-reduction assuming that reduction rate coefficients either increase with depth or are constant. Redox potential measurements from this soil were indicative of suboxic conditions at a depth of 1 mm, and stable anaerobic conditions beyond the 3 mm depth (Fig. 3A). This redox profile suggested that the effective 1^{st}-order reduction rate coefficient increased with distance in a manner that could be approximated by an arctangent function of depth (Fig. 3A). This approximation resulted in a fair match to the observed Cr profile (Fig. 3B), indicating that reductant concentrations increased with distance. Aqueous Fe(II) is likely to be the primary reductant[14,15], but its concentration profiles were not obtained. In contrast, fronts obtained using constant reaction rates (1^{st}-order rate of $3x10^{-3}$ s^{-1} shown) remain diffuse[19] and do not resemble our measured Cr profiles.

The second stage of experiments was designed to test for Cr redox zonation during contamination of natural, intact soil aggregates. Measured Cr distributions in natural aggregates (Fig. 4C) are very similar to those obtained in the model synthetic aggregates used in our earlier study. Some variability in Cr distributions is expected from heterogeneity (local mineralogy, porosity, diffusivity, redox) within and among these natural aggregates. Nevertheless, aggregates with higher available organic carbon took up higher amounts of Cr(VI), and reductively precipitated Cr(III) within shorter distances. Micro-XANES analyses showed that transported Cr was largely reduced to Cr(III), with only 23, 11, and 4% remaining as Cr(VI) in the +0, +80, and +800 ppm organic carbon systems, respectively. Chromium diffused into 100%, 80% (±7%), and 50% (±5%) of the available soil volume in the aggregates with 0, +80 ppm, and the +800 ppm organic carbon, respectively. This amounted to increases in average Cr concentrations of 212 (±20), 406 (±60), and 1,350 (±140) ppm Cr, in the contaminated regions of the 0, +80 ppm, and the +800 ppm organic carbon aggregates, respectively.

Total microbial biomass (as measured by PLFA and by direct fluorochrome staining) was not significantly affected by exposure to Cr or by differing concentrations of organic carbon. The proportion of the total microorganisms that was active however, was higher in aggregates that received the highest concentrations of organic carbon. This suggests that the spatial distribution of microbial activity in the aggregates is related to the creation of redox gradients and to Cr(VI) reduction. This was further supported by the measurement of dehydrogenase activity within the aggregates. The average dehydrogenase activity was highest in the outermost (0-20 mm) regions of aggregates presaturated with 800 ppm organic carbon (dehydrogenase concentrations of 686 μg/g soil after 7 days incubation). In contrast, deeper parts of these same aggregates (the 20-60 mm depths) had average dehydrogenase concentrations that were no higher than 17% that of outer regions.

Our proposition that larger scale subsurface behavior of contaminants is determined by biogeochemical dynamics within aggregates has at least two related precedents: the anaerobic microsite model for denitrification, and the Thiele model of porous pellet catalyst efficiency. Nitrate reduction can occur within interior regions of soil aggregates, even when aggregate surfaces are nearly in equilibrium with atmospheric oxygen, when the rate of diffusive oxygen supply can not keep up with microbial community respiration rates[3,20,21]. Besides nitrogen, the coupling of microbial respiration, diffusion limited oxygen supply, and intra-aggregate redox zonation has relevance to the fate of other redox-sensitive elements including chlorinated organics and trace element contaminants[17,22,23]. Given sufficiently large diffusion distances and respiration rates, the full spectrum of redox conditions relevant to contaminant transformations in some field sites can be contained within individual soil aggregates.

Similar diffusion-reaction problems were analyzed to optimize the efficiency of porous pellet catalysis within packed bed reactors[24,25]. Thiele modulus, Φ, is a dimensionless parameter useful in deducing when the efficiency of a catalytic pellet is diffusion limited or reaction rate limited. In environmental systems, Φ has been used in studies of denitrification in aggregated soil[26] and toluene degradation in biofilms[27]. For systems that can be approximated with first-order reaction rates and constant diffusivities, the Thiele modulus is defined by

$$\Phi = R\sqrt{\frac{k}{D_e}} \qquad (1)$$

where R is the radius of the pellet (aggregate), k is the first-order rate constant, and D_e is the effective diffusivity of reactants in the pellet (aggregate). Reactions during flow through columns consisting of pellets (aggregates) are limited by kinetics or diffusion, depending on whether Φ is small (< 0.3) or large (> 3), respectively. Effective diffusivities of most aqueous phase solutes in saturated soils are in the range of about 2 $x10^{-4}$ to 5 $x10^{-4}$ mm^2 s^{-1}. Given such diffusivities, Thiele analyses indicate that transformations in aggregates involving k values greater than about 10^{-4} s^{-1} and diffusion distances greater than about 5 mm are either influenced or determine by diffusion rather

than by reaction rates. For Cr(VI), its slow diffusion into reducing regions can result in very localized Cr(III) precipitation fronts, beyond which Cr transport is prohibited.

Chromium contamination within soil aggregates can be strongly diffusion-limited, resulting in reduction to Cr(III) within short distances. In large diffusion-limited domains, the Cr-contamination can be restricted to outer regions in contact with preferential flow paths, leaving the deeper core region unaffected. Such aggregates contain microbial communities that have and have not been exposed to Cr(VI) residing within outer and core regions, respectively. These results show the importance of intra-aggregate spatial relations for redox sensitive contaminants as well as for the microbial communities responsible for redox gradients and reductants. By extension, similar stratification of redox potentials, metal contaminants, and microbial communities might occur within larger sediment blocks deeper in the subsurface. In soils and sediments comprised of aggregates or blocks that support internal redox gradients, bulk characterization of chemical and microbiological conditions does not allow mechanistic understanding of biogeochemical processes controlling the fate and transport of contaminants.

Methods

Column experiments

In the column studies, experiments on Cr diffusion and reduction were conducted in 30 mm long soil columns. This soil, an Aridic Haploxerert (Altamont Pass, Alameda County, CA), is calcareous (0.8 to 4.2% $CaCO_3$, pH 8.5), and 40% to 55% clay (primarily as smectite and kaolinite). Aggregates from the C horizon were crushed, homogenized, and repacked in the first series of experiments, and used intact in the second series. Prior to introducing Cr(VI), columns were saturated with solutions containing from 0 to 800 ppm organic carbon, and incubated for 14 days. Tryptic soy broth was used as the organic carbon amendment to simulate natural subsurface organics of plant origin. All incubations were done at room temperature. The carbon amendments were added to stimulate microbial respiration and develop more reducing conditions. Cr(VI) solutions were then introduced to one end of each column (representing the exterior surface of an aggregate), and allowed to diffuse inwards. Redox potentials were measured at 2 to 5 mm depth increments with Pt electrodes. The GeoSoilEnviroCARS beamline 13ID-C at the Advanced Photon Source (Argonne, IL) was used to obtain micro x-ray absorption near edge structure (micro-XANES) spectroscopy profiles of Cr(VI) and Cr(III) distributions[28]. Bacterial communities in these systems were characterized using DNA fingerprints. The conserved bacterial primers 27F and 1492 were used to generate the community fingerprints (18).

Intact soil aggregate experiments

In order to prevent breaking during wetting, these large (90 to 150 mm diameter) aggregates were wrapped in cheesecloth, and supported in coarse sand. The same solutions used in the previous experiments (0, 80, and 800 ppm carbon as tryptic soy broth) were used to wet these soil aggregates. Following 17 days of incubation, these

aggregates were immersed in individual containers of Cr(VI) solutions (1,000 ppm initial Cr) for 3 days, representing an episodic contamination event (Fig. 4A). After removal from Cr(VI) solution, aggregates were either immediately frozen or incubated for an additional 31 days (room T), then frozen (-70˚C), freeze-dried, fixed with a low viscosity resin (LR White resin, London Resin Co. Ltd., Berkshire, England), and cut to obtain 5 mm thick slab cross-sections (Fig. 4B). One surface of each slab was polished for synchrotron x-ray microprobe mapping of Cr distributions. An additional set of aggregates was prepared in the same manner, without freezing and fixing with resin. Each of these aggregates was cored to characterize depth profiles of microbial communities (Phospholipid Fatty Acid (PLFA) analyses, enzyme analyses, and the density and activity of total microorganisms as measured by direct fluorochrome staining), and for additional Cr XANES analyses. X-ray microprobe maps of Cr distributions in resin-fixed aggregates were collected in only 10% to 20% of each section, in 1 mm steps, because of their relatively large total sample area. These Cr maps were obtained at beamline X26A of the National Synchrotron Light Source (Upton, NY). A large (300 μm) x-ray beam was used to average over many individual soil particles and pores within a single point measurement. The Cr(VI) analyses were done on triplicate core samples collected from each aggregate on day 31, and refrigerated prior to their micro-XANES measurements 41 days later. Micro-XANES measurements on resin-fixed aggregates were not used to infer oxidation states because Cr(VI)-spiked, resin-impregnated control samples exhibited significant reduction artifacts.

References and Notes

1. S. Dudka, D. C. Adriano, *J. Environ. Qual.* **26**, 590 (1997).
2. W. Shotyk, D. Weiss, P. G. Appleby, A. K. Cheburkin, R. Frei, M. Gloor, J. D. Kramers, S. Reese, W. O. Van Der Knapp, *Science* **281**, 1635 (1998).
3. J. A. Currie, *Soil Sci*. **92**, 40 (1961).
4. W. Wilcke, M. Kaupenjohann, *Geoderma* **83**, 55 (1998).
5. A. G. Seech, E. G. Beauchamp, *Soil Sci. Soc. Am. J*. **52**, 1616 (1988).
6. M. Drazkiewicz, *Folia Microbiol*. **39**, 276 (1994).
7. D. M. Proctor, B. L. Finley, M. A. Harris, D. J. Paustenbach, D. Rabbe, *Chromium in Soil: Perspectives in Chemistry, Health, and Environmental Regulation* (Lewis Publishers, Boca Raton, FL, 1997).
8. D. Rai, L. E. Eary, J. M. Zachara, *Sci. Total Environ.* **86,** 15 (1989).
9. B. R. James, *Environ. Sci. Technol*. **30**, 248A (1996).
10. D. R. Lovley, *Annu. Rev. Microbiol.* **47**, 263 (1993).
11. M. E. Losi, C. Amrhein, W. T. Frankenberger Jr., *Environ. Toxicol. Chem*. **13**, 1727 (1994).
12. D. R. Lovley, E. J. P. Phillips, D. J. Lonergan, *Environ. Sci. Technol.* **25**, 1062 (1991).
13. F. H. Chapelle, *Ground-water Microbiology and Geochemistry*; (John Wiley and Sons, New York, 1992).
14. S. Fendorf, B. W. Wielinga, C. M. Hansel, *Int. Geol. Rev*. **42**, 691 (2000).

15. Wielinga, B., M. M. Mizuba, C. M. Hansel, S. Fendorf, *Environ. Sci. Technol.* **35**, 522 (2001).
16. M. E. Losi, W. T. Frankenberger, Jr., *Water Air Soil Pollut*. **74**, 405 (1994).
17. T. K. Tokunaga, J. Wan, M. K. Firestone, T. C. Hazen, E. Schwartz, S. R. Sutton, M. Newville, manuscript submitted to *Environ. Sci. Technol*.
18. W.T. Liu, T.L. Marsh, L.J. Forney, Appl. Environ. Microbiol. **63**, 4516 (1997).
19. J. Crank, *The Mathematics of Diffusion*, 2nd Edition (Clarendon Press, Oxford, 1975).
20. K. A. Smith, *Soil Sci*. **123**, 284 (1977).
21. J. Zausig, W. Stepniewski, R. Horn, *Soil Sci. Soc. Am. J*. **57,** 908 (1993).
22. M. V. Enzien, F. Picardal, T. C. Hazen, R. G. Arnold, C. B Fliermans, *Appl. Environ. Microbiology* **60,** 2200 (1994).
23. T. K. Tokunaga, S. R. Sutton, S. Bajt, *Soil Sci*. **158**, 421 (1994).
24. E. W. Thiele, *Ind. Eng. Chem.* **31**, 916 (1939).
25. P. B. Weisz, *Science* **179**, 433 (1973).
26. D. D. Myrold, J. M. Tiedje, *Soil Sci. Soc. Am. J.* **49**, 651 (1985).
27. P. A. Holden, J. R. Hunt, M. K. Firestone, *Biotechnol. Bioeng*. **56**, 656 (1997).
28. D. G. Schulze, P. M. Bertsch, *Adv. Agron*. **55**, 1 (1995).

Acknowledgments
We thank Robert Giauque, Tracy Letain, and Andrew Mei of LBNL, and GSECARS staff for assistance, and Harvey Doner (University of California, Berkeley) for internal manuscript review. Funding was provided through the U. S. Department of Energy, Natural and Accelerated Bioremediation Research Program, and Basic Energy Sciences, Geosciences Program. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science. Research carried out (in part) at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the U. S. Dept. of Energy, Division of Materials Sciences and Division of Chemical Sciences.

Correspondence and requests for materials should be addressed to T.K.T. (e-mail: tktokunaga@lbl.gov).

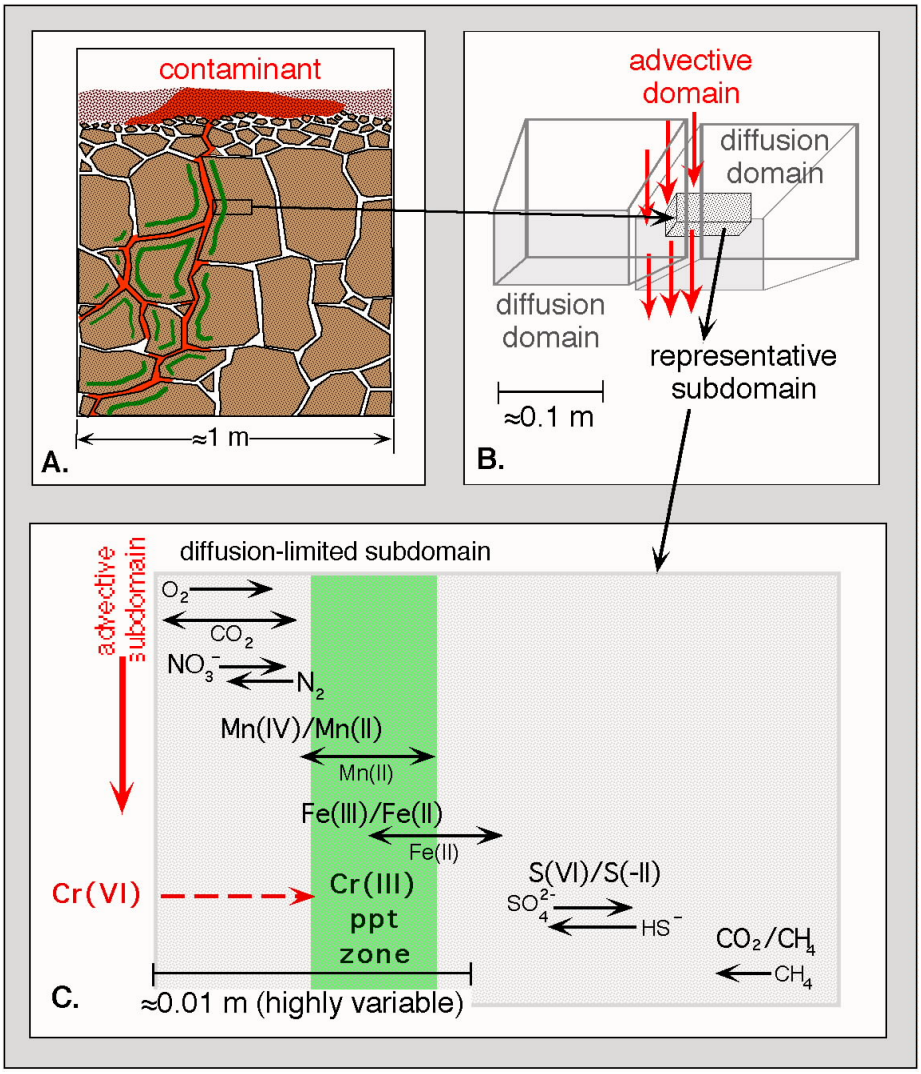

Figure 1. Conceptual model of flow and transport in structured subsurface environments. **(A)** Most of the flow occurs within the advective domain, which is often a small fraction of the total system volume. **(B)** The remaining, often larger, fraction of the subsurface exchanges chemical species primarily through diffusion. **(C)** Microbial activity can cause large gradients in redox potentials within these diffusion-limited domains and localized precipitation of redox-sensitive contaminants.

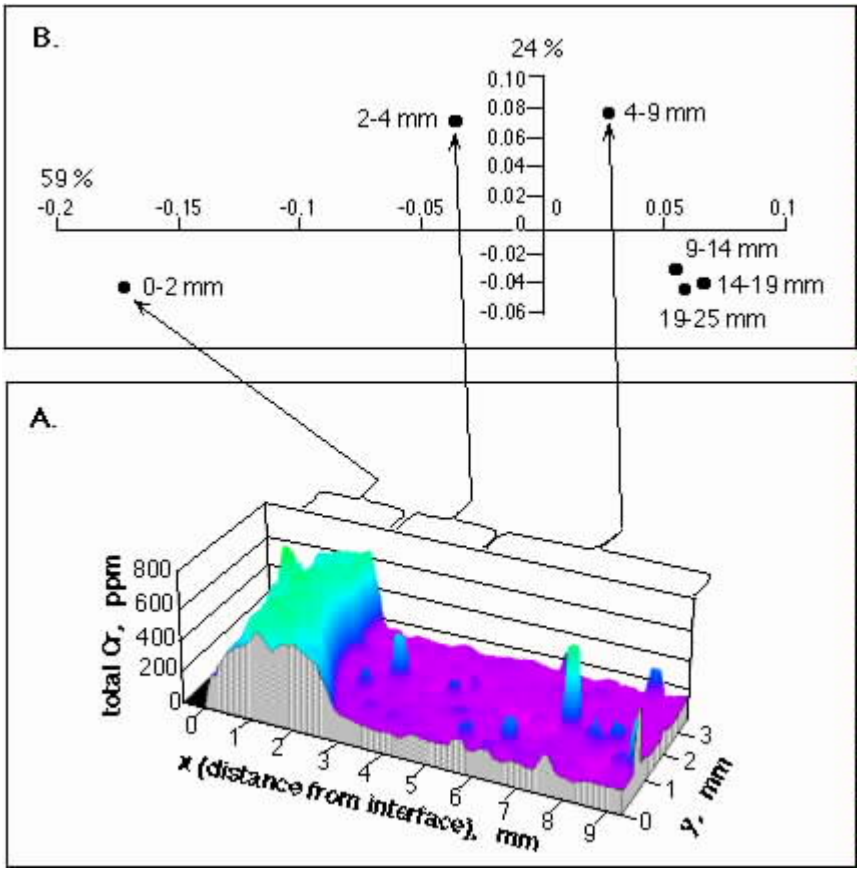

Figure 2. (**A)** X-ray microprobe map of total Cr in a synthetic soil aggregate, pre-incubated with 80 ppm organic carbon solution, and exposed to a pool of 260 ppm Cr(VI) for 2.5 days. Greater than 95% of the contaminant was reduced to Cr(III) within 2 mm of the exposure surface. The native soil Cr is essentially all Cr(III), with an average concentration of 150 ppm, but heterogeneously distributed (note Cr map at depths greater than about 2 mm). **(B)** Principal component plot of bacterial terminal restriction fragment length polymorphism (TRFLP) patterns from the synthetic soil aggregate.

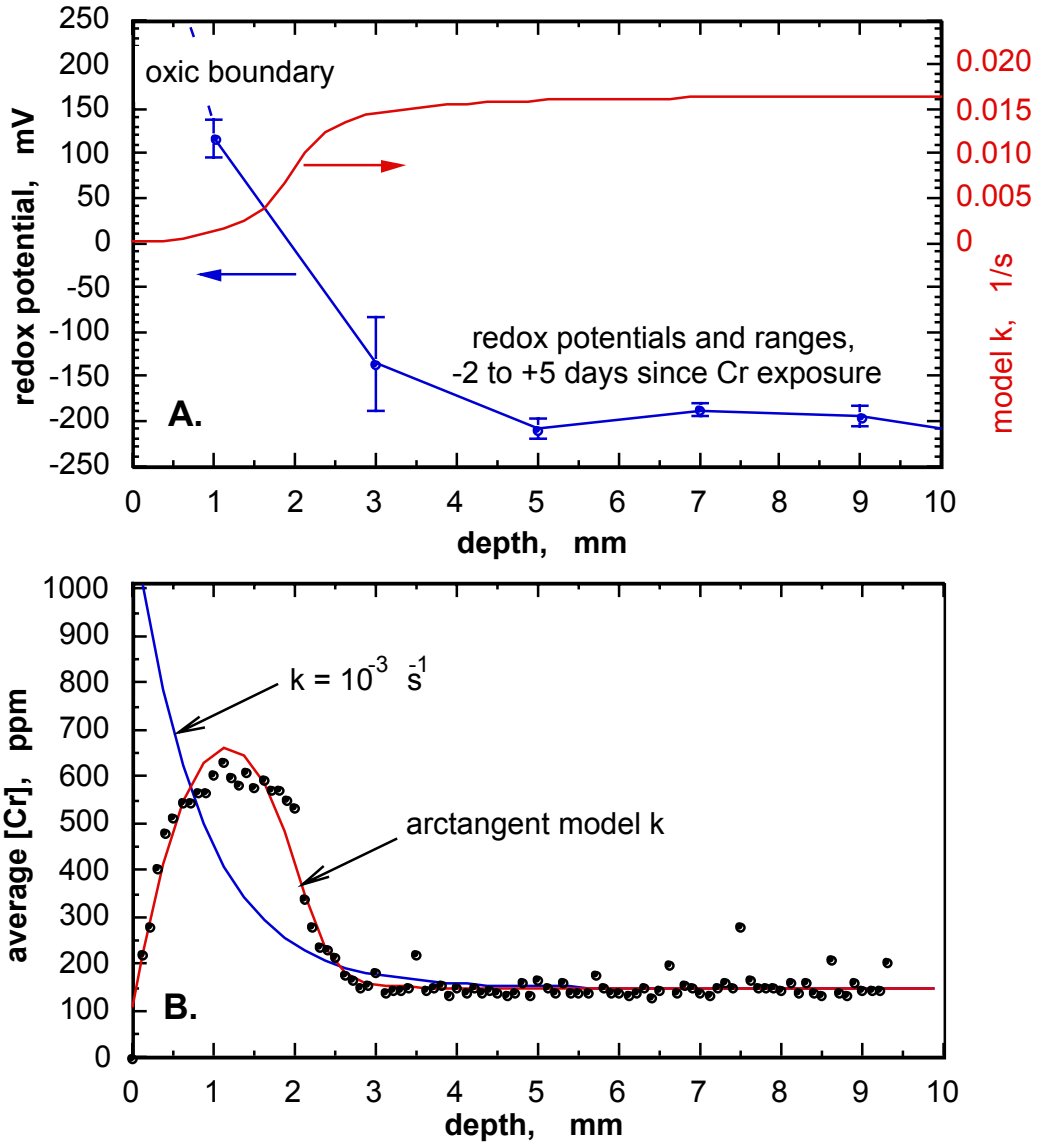

Figure 3. (A) Redox potential profiles from the soil shown in Fig. 2A, and arctangent depth dependent 1st-order rate used to model Cr diffusion-reduction. **(B)** Comparison of the average Cr depth profile from Figure 2A with model calculations assuming either spatially-dependent or fixed 1st-order Cr(VI) reduction rate constants. The precipitation of Cr(III) within a narrow zone, and with an abruptly terminated front, is reproduced with the increasing reduction rate constant model.

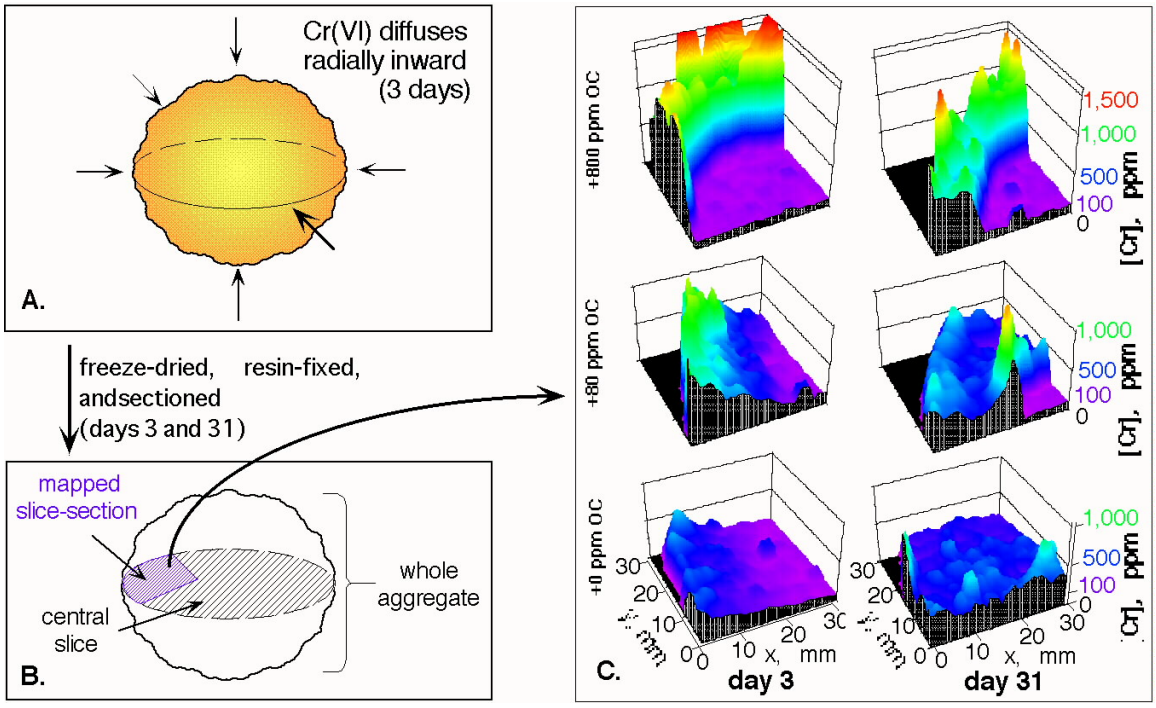

Figure 4. Synchrotron x-ray microprobe maps of total Cr in sections from 6 soil aggregates that were exposed to Cr(VI). Each aggregate was incubated for 17 days (room T) with either +0, +80, or +800 ppm organic carbon (as tryptic soy broth) solutions, then exposed to 1,000 ppm Cr(VI) solutions for 3 days. The native soil [Cr] = 120±40 ppm, and the black region is outside of the aggregate. The three aggregates on the left were freeze-dried on the 3rd day, while those on the right were incubated (room T) for an additional 28 days (removed from Cr(VI) reservoir), then freeze-dried. The more reducing conditions established in the higher organic carbon aggregates result in more rapid Cr(VI) reduction at shorter distances, higher diffusive uptake of Cr, and sharply terminated contaminant fronts. Thus, highly contaminated and uncontaminated region can coexist within aggregates and sediment blocks at contaminated sites.